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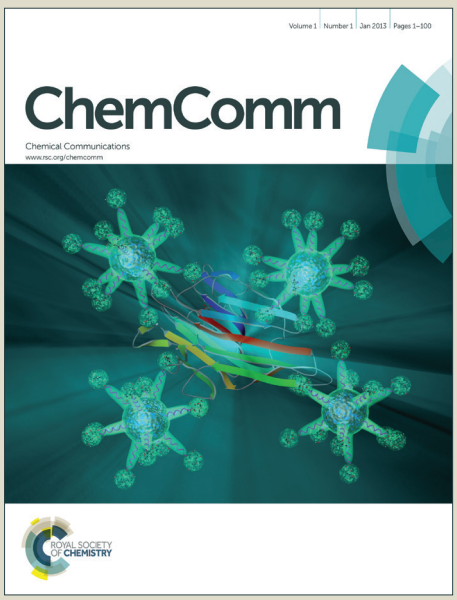

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Sensitive fluorescent and colorimetric detection of ATP based on a strategy of self-promoting aggregation of a water-soluble polythiophene derivative

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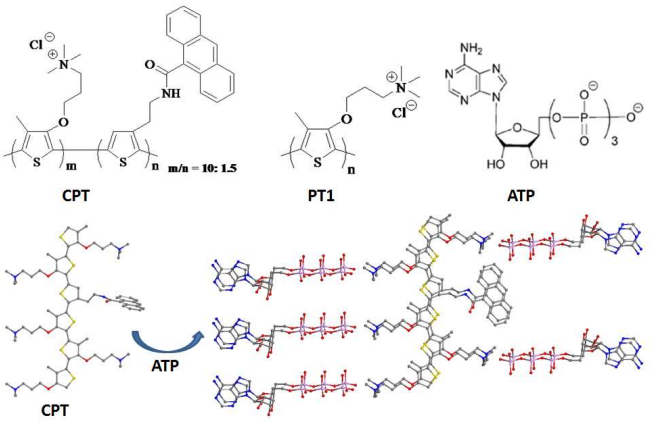
A sensitive fluorescent and colorimetric dual-modal probe for the detection of ATP have been developed based on a strategy of self-promoting aggregation of a cationic polythiophene derivative bearing anthracene groups in the side chain with a detection limit as low as 10^{-9} M range.

The detection of adenosine triphosphate (ATP) has drawn considerable attention in recent years,[1] because ATP plays important roles in cell biology.[2-4] Visible detection and imaging of ATP can facilely offer useful information about the production and consumption of ATP in real time.[5] Accordingly, a number of fluorescent or colorimetric sensors for ATP have been designed and reported.[6] However, It remains a challenge to find new approaches that could improve the simplicity, selectivity, and sensitivity of ATP detection.

Recently, a new sensory material based on water-soluble polythiophene (PT) derivatives have received extensive attention because of their sensitive chain conformation to external stimuli,[7-12] providing an unique potential for colorimetric and fluorometric dual-output. Up to date, several kinds of cationic PT derivatives have been synthesized and applied to be colorimetric and fluorescent probes for the detection of DNA and proteins,[12e,13] monitoring the helicity and conformational transition in polysaccharides,[14] and sensing small bioanions such as nucleotides,[6i,7a] folic acid and so on.[15-17] It is easy to find that various biomacromolecules, such as DNA and protein, can bind strongly with PT chains, providing efficient colorimetric and fluorescent signal outputs, whereas small analytes with simple structures cannot induce distinct conformational changes and aggregation of PT-based probes due to the relative weak interactions between analytes and PT chains, resulting in the weak optical outputs. To improve the sensitivity for sensing some small anions with PT-based probe system, a strategy based on the premodification of analytes to increase the interactions between analytes and probe has been developed.[16-18] Herein, we develop a sensitive fluorescent and colorimetric dual-modal probe for the detection of ATP by taking advantage of a strategy of self-promoting aggregation of a cationic copolymer **CPT** bearing anthracene groups in the side chain. The overall strategy is illustrated in Scheme 1. Upon mixing, electrostatic interactions bring together the optical probe **CPT** and ATP. The spatial constraints with in **CPT**/ATP aggregates force the **CPT** adopt a more planar conformation, subsequently resulting in a aggregation mode of PT backbones. During this process, anthracene groups with large aromatic plane in the side chain of **CPT** will promote the polymer aggregation through interchain π-π stacking interaction to provide more sensitive sensing for ATP compared with the cationic PT1 system (Scheme 1). Attempts have also been made to open a new and general way for improving the sensitivity of PT-based probes for small molecules as well as to understand the copolymer **CPT**/ATP supracomplex formation by the use of different techniques and through fine-tuning experimental conditions.



Scheme 1 Schematic illustration of the detection of ATP based on a self-accelerating aggregation of **CPT**.

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Copolymer probe **CPT** was synthesized by the chemical oxidative polymerization with $FeCl_3$ in chloroform of 3-(4-Methyl-3'-thienyloxy)propyltrimethyl-ammonium bromide with 4-[9-anthrylcarboxy(N-methylamino)]thiophene,[14a,19] Synthetic details are presented in the ESI.

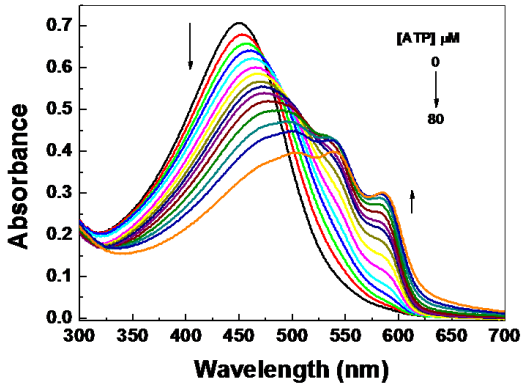

Fig. 1 UV-vis titration spectra of **CPT** (100 μM) in tris-HCl buffer solution with increasing amounts of ATP. [ATP] = 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 52, 60, 68, 80 μM from top to down.

Titration of **CPT** with ATP in tris-HCl buffer solution (2 mM, pH 7.4) at 23 °C was monitored by absorption spectroscopy. As shown in Fig. 1, the absorption maximum of **CPT** appears at around 450 nm (ε = 7.08 × 10^3 M^{-1} cm^{-1}), which is associated to the π-π* transition of random-coiled polymer chains. Upon adding increasing amounts of ATP, the absorption maximum is gradually red-shifted from 450 to 588 nm together with solution color change from yellow to deep violet. The remarkable red-shift (by 138 nm) and the appearance of characteristic vibronic bands at 540 and 588 nm were related to the changes in the conformation from nonplanar-to-planar and aggregation mode of PT backbones as demonstrated by dynamic light scattering (DLS) (Fig. S1, ESI). It was noted that titration reached saturation at [ATP] = 80 μM (0.8 equiv), which is far below that in the PT1 system (5 equiv) in water,[6i] signifying that aggregation of **CPT** chains were facilitated upon noncovalent binding with ATP which is probably related to the interchain π-π stacking interaction between anthracene groups (Scheme 1). Benesi-Hildebrand analysis of the absorption changes (Fig. S2, ESI) showed a near 1:1 stoichiometry for the complex formed between **CPT** and ATP with a binding constant of K_{assoc} = 7400 M^{-1} in buffer solution.[20]

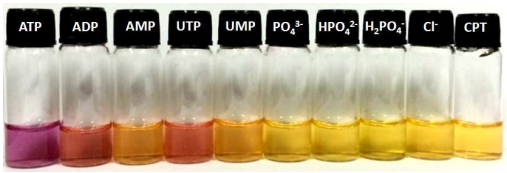

Fig. 2 Changes in the color of solutions of **CPT** (100 μM) in water induced by the addition of an equimolar amount of various anions.

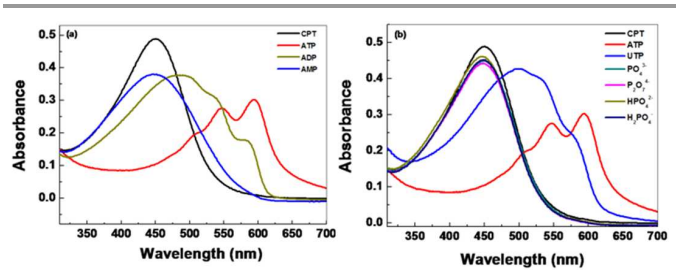

Fig. 3 Absorption spectra of **CPT** (100 μM) in water in the absence of anionic guest and (a) in the presence of an equimolar amount of ATP, ADP, or AMP, or (b) in the presence of an equimolar amount of ATP, UTP, or phosphate.

To address the molecular mechanism beyond this observation, the influence of the nucleotides those bear different numbers of phosphate groups (negative charges) and different nucleobase moieties on the polymer aggregation was examined. The changes in the absorption spectra of **CPT** in water upon addition of biologically important anions such as ADP, AMP, UTP, UMP, as well as chloride and various phosphate ions (as sodium salts) were shown in Fig. 2 and Fig. 3. As shown in Fig. 2, after addition of an equimolar amount of these anions to aqueous solutions of **CPT**, most of the solutions remained yellow with λ_{max} < 450 nm except for those that contained ADP and UTP, which gave amber color solutions with shifts of the absorption maximum to about 580 and 500 nm associated with vibronic bands, respectively. The most remarkable effect was noted, however, upon addition of ATP, which gave a deep violet solution (Fig. 2). The dramatic color change of **CPT** upon addition of ATP provides a very simple means for naked-eye discrimination of ATP from various nucleotides in aqueous solution. From Fig. 3a, the largest absorbance response of aqueous **CPT** solution was observed upon the addition of an equimolar amount of ATP, whereas unconspicuous changes occurred upon blending with adenosine monophosphate (AMP), suggesting that the amount of negative charge on the analyte plays a crucial role in the polymer aggregation. However, only the presence of inorganic polyphosphate ion does not induce a distinct change in the color of the solution of **CPT** and no supramolecular aggregate is formed in the mixture (Fig. 3b). Thus, it is very important to consider the effect of different nucleotides on the aggregation of **CPT**. As shown in Fig. 3b, relatively smaller spectral changes were observed upon exposure of **CPT** to uridine triphosphate (UTP) and various inorganic phosphates, indicating that the nucleobase adenine with a larger aromatic plane facilitated the polymer aggregation through hydrophobic effects.

We then examined the circular dichroism (CD) spectral changes of **CPT** in water with a variety of nucleotides (Fig. S6, ESI). **CPT** itself is optically inactive, and no CD pattern in the π-π* transition region was detected, which indicates that **CPT** adopts an achiral random-coiled conformation in water. Upon binding with ATP, an intense split-type induced CD (ICD) in the π-π* transition region was observed, suggesting the formation of chiral superstructures between **CPT** and ATP.[12b]

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In addition, the ICDs intensities increase gradually with increasing the concentrations of ATP (Fig. S7, ESI).

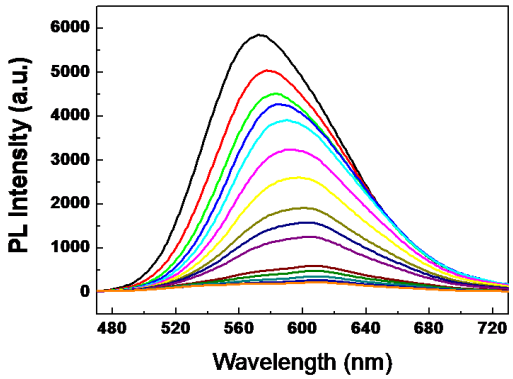

Fig. 4 Fluorescent titration spectra of **CPT** (100 μM) in tris-HCl buffer solution with different concentrations of ATP. [ATP] = 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 30, 40, 50, 70, 90 μM from top to down. λ_{ex} = 450 nm.

Fluorescent titration of **CPT** (100 μM) with ATP in tris-HCl buffer solution led to a dramatic quenching of the emission of **CPT** at 570 nm (λ_{ex} = 450 nm), signifying the possibility for constructing colorimetric and fluorescent dual mode chemosensor for the detection of ATP. As shown in Fig. 4, free **CPT** chains in tris-HCl buffer solution with random-coiled conformation exhibits an emission band around 570 nm upon excitation at 450 nm (Φ = 0.304). Upon addition of increasing amounts of ATP, the emission intensity decreases gradually with a obvious red-shift and broadening of the band is observed, which indicates the fluorometric detection of ATP binding is possible. The relative weak emission observed from aggregates as compared to that of free chains is a result of a more efficient radiationless decay in the ordered phase. The fluorescence quenching of **CPT** is very sensitive to binding with ATP, and a near 100 % quenching at the emission maximum was observed in the presence of an equimolar amount of ATP, whereas only 28 % and 15% quenching of the fluorescence was detected upon addition of an equimolar amount of ADP and AMP, respectively (Fig. S8, ESI). These results indicate that the quenching of fluorescence is much more effective in the presence of ATP than with the use of other nucleotides such as ADP, AMP, UTP and UMP. Fluorescence quenching efficiencies can be quantified by the use of the Stern–Volmer equation (Fig. S12, ESI). The Stern–Volmer constant, K_{SV}, determined from the linear portion of a plot of F_0/F_i versus [ATP] is of 4.46×10^5 M^{-1} (R = 0.994). The detection limit, based on fluorescence quenching, for ATP was estimated to be 2.3×10^{-9} M.[21]

In conclusion, we have developed a sensitive colorimetric and fluorescent dual-response probe for the detection of ATP at physiological pH conditions based on a strategy of self-promoting aggregation of a PT derivative. Although further research is still required to understand the sensing mechanism behind the better selectivity for ATP, these results have indicated that a tiny and subtle adjustment of the structure of PTs can efficiently improve the sensitivity of PT-based probe toward a special analyte by controlling the interplay between side-chain steric/electrostatic repulsive and interchain attractive interactions of PTs. This strategy is expected to be applied to other conjugated polyelectrolyte (CPE)-based sensing system.

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Notes and references

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